

The University of Chicago

MECHANISM OF THE EFFECT
OF HYPERTHYROIDISM ON
CARDIAC GLYCOGEN

A PART OF A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CAN-
DIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

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The cardiac manifestations of hyperthyroidism—hypertrophy, pathological changes, and chemical alterations—have been attributed by some investigators to a specific “toxic” action of the thyroid hormone, by others to purely physical and physiological phenomena; the proponents of neither viewpoint have made any serious attempt at an experimental approach to the problem’s resolution. This seems attainable most decisively by utilizing as test object a factor which can be quantitatively analyzed, which is altered significantly by hyperthyroidism, and which is not so altered by any disease or other uncontrollable process which might enter the picture. Such a test object is the glycogen content of the heart.

The earliest discoverable report that cardiac glycogen is diminished in hyperthyroidism appeared in a remark by Goodpasture (1) that he could discern no microscopically identifiable glycogen in the hearts of chloroformed, hyperthyroid rabbits. He did not make clear whether a similar loss was also to be found in hyperthyroid animals which had not been subjected to chloroform. Hoet and Marks (2) made the first definite statement, based on chemical analyses, that heart muscle was depleted of its glycogen content by thyroid feeding, and this conclusion has been repeatedly verified by many independent investigators.

The only other factor which has been found uniformly to elicit a loss of cardiac glycogen is a diminished oxygen supply. Thus, Evans (3) found that hypoxia depressed the heart’s concentration of glycogen in parallel with the extent to which oxygen was replaced by nitrogen. Similarly, it has been recognized for many years that tissues intended for glycogen analysis must be treated as rapidly as possible to stop glycogenolysis after arrest of the oxygen supply, and a major source of error has resulted from all-too-frequent disregard of this precaution.

That the glycogen depletion of the hyperthyroid heart is probably not accountable to avitaminosis B₁ is indicated by the repeated finding, most recently reported by Edlund and Holmgren (4), that severe thiamin deficiency does not lower cardiac glycogen. As a matter of fact, it is unlikely that the intensity and duration of hyperthyroidism induced in the present investigation is such as to cause any significant avitaminosis.

The decrease in heart glycogen resulting from the hyperthyroid state may be considered due either to a specific “toxic” effect or to a derangement in one or more physiological mechanisms as a result of the alteration of cardiac activity. If it is a consequence of increased activity of the heart, whether related to an augmented intensity of metabolism or work performance, or even to the increased

¹ Dissertation for Ph.D. degree in the Department of Physiology.

heart rate *per se*, then a similar loss of heart glycogen should be elicitable by chronically increasing cardiac activity through non-thyroid means. The present investigation is an attempt to stimulate the non-hyperthyroid heart, to depress activity of the hyperthyroid heart, and to analyze the effects on heart glycogen in the light of known physiological mechanisms.

METHODS. Male adult albino rats were used throughout. Although litter-mate controls were established in each experiment, it was not found necessary to separate the data, for the variations in control heart glycogens within any litter were no greater than those in the colony as a whole.

The basal ration consisted of Purina dog chow, *ad libitum*. This apparently led, at one stage, to a dietary deficiency resembling mild manganese-lack. It was soon eliminated by addition of weekly portions of lettuce, but not by carrots or cabbage. Subsequently, the lettuce supplement was replaced by the addition of about 10 ppm. of $MnCl_2$ to the drinking water. Under this regime, fertility was normal, fur and eyes appeared healthy, and growth was rapid, attaining a weight of well over 300 grams at six months.

During, and for at least three months before, the experimental period, the animals were housed in a constant temperature room at about 27°C.

Determination of heart glycogen. The method of preparing the heart for the glycogen analysis proved a major factor in obtaining consistent results. After a 24 to 36-hour fast, the unanesthetized rat was smartly transected somewhat above the level of the heart by a single cut with a large tinsmith's bench shear. Manual compression of the lower chest then exteriorized the heart, so that a single snip with scissors could drop it, preferably still attached by the pulmonary vessels to sufficient lung tissue to ensure flotation, into a container of vigorously boiling Ringer's solution. Time lapse between transection of the animal and destruction of glycolytic enzymes usually amounted to 1 to 3 seconds; actually, as many as 10 to 15 seconds could be lost in the operation without significant destruction of glycogen.

It was found necessary to use a fresh supply of boiling Ringer's solution for each heart.

The boiled heart was transferred to a shallow dish of Ringer's solution at room temperature, where it was carefully dissected free of all non-cardiac tissue. It was found advisable to eliminate the atria as well; otherwise, a variable amount of vascular tissue would have remained with the heart. The ventricles were cut into three or four portions, such that the chambers could be thoroughly freed from blood, and each segment was carefully blotted on filter paper, weighed on the torsion balance, and dropped into 2 cc. of 30 per cent KOH for the preliminary digestion.

The purification and hydrolysis of glycogen followed the procedure of Good, Kramer and Somogyi (5), and the glycogen hydrolysate was analyzed for glucose by Somogyi's (6) modification of the Shaffer-Hartmann method. The glycogen value was derived from the product of "glucose obtained" times the factor 0.927 and was in all instances converted to milligrams per cent.

As a result of the elimination of blood and non-cardiac tissues, the values obtained proved higher and more consistent than any to be found in the literature. The average, and standard deviation, on 20 normal rats was 659 ± 51 mgm. per cent. Extremes were 744 and 526, but since the latter value was the only one below 600 mgm. per cent, it may be assumed faulty, though not discarded. The individual values have been incorporated into graph *d*, figure 2.

Measurement of heart rate. Methods of counting the heart rate of the rat during forceful restraint, especially by needle electrodes, could not be expected to obtain even approxi-

mately basal values in these highly excitable subjects, and those involving palpation or auscultation appeared too prone to subjective variance. The procedure developed for the present investigation involved registration of the electrocardiogram from the surfaces of the animal's feet. At first, this was transcribed with the usual electrocardiograph, but, aside from the expensiveness of bromide paper and the time lost in developing the film, the machine was disadvantageous in that it failed to tap the heart's potential through the callouses which developed on the rat's feet as a result of friction against the false bottom of its cage. A change was made, therefore, to the apparatus devised by Gerard and his associates for the recording of brain waves. By this means, the impulse was picked up by contact electrodes, stepped up by Offner's (7) amplifier, and inscribed by the piezo-electrode crystograph of Offner and Gerard (8) on adding machine tape. Since the tape was moved by a constant speed motor, the heart rate could be measured with great accuracy by the use of dividers.

Construction of the animal holder is indicated in figure 1. Copper plates imbedded in the hard rubber floor served as pick-up electrodes, one for both forefeet and the other for the hind feet. Soldered to each plate was a long iron screw which pierced the floor and dipped into a mercury cup set into the shelf on which the cage rested. The potentials were thus led into the amplifier and crystograph. Animal, cage, and wiring were shielded from external interference by a copper mesh screen; such interference was frequently utilized as a time check by simply opening the screen door and taking a record of the 60-cycle hum. Neither electrode paste nor saline was required to facilitate transmission.

To simulate the rough floor to which rats apparently are partial, a square of copper screening was soldered to each of the copper plates. The sides and roof of the animal holder were formed by a single bent sheet of heavy celluloid. Slots cut into the celluloid provided apertures for the fastening screws at the sides of the cage floor and at the same time rendered the cage adjustable to animals ranging between 150 and 450 grams body weight.

The front gate was fashioned of heavy sheet metal with a fairly large copper screen window for ventilation. A $1\frac{1}{2}$ -inch overhang was bent into the upper part of the front gate to shade the animal's head. (This author finds no merit to the almost universal assumption that bright light induces rats to sleep; it probably irritates their eyes, which are minimally protected by pigment, and forces the animals to close them and to curl up for shade. It was observed, in fact, that the animals were quieter when the screen on the front gate was coated with flat black paint.)

A large slot was cut into the tail gate to allow protrusion of the tail and to avoid pinching the scrotum. It was also found advisable to use a small plate to close this slot in those instances, early in the training period, when a rat succeeded in turning around in the cage; without such a device, he was likely to gnaw at the edge of the tail gate.

With this set-up it was nevertheless found necessary to allot at least a month for training, before the recorded heart rates reached a plateau. This was done by placing about a dozen rats at a time into separate animal holders and simply leaving them for about half an hour every day.

The heart rate recorded for each run was the lowest one that was found in a ten-minute tracing, made after a ten-minute rest. Any bodily movement could be easily detected by irregular oscillations of the crystograph pen. It soon became clear, however, that physical activity was not the only factor causing variations in heart rate. In most subjects, neither the final "basal" rate of each record nor the rates obtained on different days jibed as completely as one might predict they would with the state of rest or activity. Since a spontaneous quickening of the pulse often anticipated a movement and since activity was in other instances neither accompanied nor followed by cardio-acceleration—sometimes, even, by a deceleration—it became patent that greater variation was induced by the psyche than by the soma.

Temperature changes likewise exercised an influence on the heart rate. On a few occasions, cold spells which were not anticipated and prepared for succeeded in lowering

the room temperature by several degrees. All heart rates taken during such periods were greatly elevated.

In assigning normal heart rates for comparison with those attained during the experimental period, each animal's graphic record of "basal" heart rates, covering a period of 3 to 6 weeks, was scanned and a "predictable" rate adopted. The resultant values, not truly basal for more than a small proportion of the 113 male rats, ranging between 200 and 370 grams body weight, which were used, averaged 4.4 ± 0.30 per second, or 264 ± 18 per minute.

Measurement of basal metabolism. The machine devised for determination of basal metabolism was of the closed-circuit type, measuring only the oxygen consumption. Water bath, spirometer box, and animal chamber were all constructed of sheet brass, which proved adequate for rapid equalization of temperature throughout the system. This temperature was maintained within narrow limits by means of a tubular model heating unit, controlled through a relay by a thermoregulator immersed in the water bath. The water was circulated by a noiseless electric stirrer. Effectiveness of the temperature control was checked by observing the time consumed in reaching constant volume after filling the system with oxygen; this process never required more than fifteen minutes. Once constant, the volume did not vary more than half a cubic centimeter during the interval between successive activations of the thermal element. Temperature within the animal chamber was kept at $28^{\circ}\text{C}.$, the temperature of thermic neutrality for the albino rat.

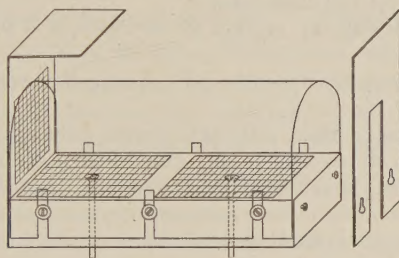


Fig. 1. Cage used in determining rat heart rate. Description in text. One-quarter scale.

The animal chamber was rendered airtight by the usual water seal. Its lid was provided with a large window made of two sheets of glass separated by an air space to limit condensation of water. The thermometer and a stopcock, to allow free movement of air into the system whenever the apparatus was opened, were fitted into the lid.

A false-bottom floor within the animal chamber was formed by the copper screen lid of a soda lime tray, whose bottom also was made of copper screening. This entire tray was elevated about an inch above the true floor of the animal chamber, leaving space for a pool of water below the CO_2 absorbent. Koehler (9) pointed out the advisability of maintaining a relatively high humidity within the chamber, partly to keep the soda lime at maximum absorptive efficiency and partly to induce the animal to relax. He considered that a vapor tension exceeding 75 per cent, however, was not thus conducive to rest. The present author, on the other hand, observed that the sedative action of humidity on rats varied directly with vapor tension and that a pool of water on the floor (99 per cent humidity at $28^{\circ}\text{C}.$) was most productive of rest. Under this regime, the animals almost immediately composed themselves for sleep, with but a small proportion of recalcitrants.

The spirometer bell was made of thin-walled brush copper, which is far less vulnerable than aluminum to attack by soda lime. Of the Krogh type, operating on the principle of a hinged box, it pivoted about jewel bearings, thus allowing complete freedom of movement without the shifting encountered in the cylinder type spirometer. The bell was counter-

weighted in such manner that, with the system opened to the outside, it descended slowly and without acceleration. At the end of the stainless steel tubular writing lever was attached a free-swinging capillary tubing writing point which exerted a constant pressure, determined by its own very slight weight, against the kymograph drum. Its tip was melted into a smooth ball to minimize frictional variation.

Use of these contrivances led to a high degree of sensitivity, so much so that the slightest movement of the rat communicated itself as gross oscillations to the kymogram. Such precision also made possible the measurement of oxygen consumption in five-minute periods. It was found that a sufficiently sensitive machine could be used for such short intervals, in actual fact gave lower and more constant results than any recording over longer periods.

In view of the fact that comparisons were to be made between the normal and experimental states in the same animal, all basal metabolism values were expressed as "cc./hr." Had body weight or surface area been used for reference, the loss of body fat with development of hyperthyroidism would have made the experimental figures inordinately high. For comparison with the data of other workers, however, the values were also converted for expression in the most customary terms. Of 132 determinations, during sleep, on 40 rats, the following equivalent averages were obtained:

686 cc./kgm./hr. (S. D., ± 45 cc.)

79.4 Cal./kgm./day

594 Cal./sq. m./day (S. = $kW^{2/3}$; $k = 9.1$)

These animals, all males, were 6 to 8 months old and ranged in weight from 267 to 370 grams (average, 315 grams).

In actual use, it was found that, although normal values were adequately constant, rarely varying as much as 10 per cent in any individual (sleeping state), determinations made upon hyperthyroid rats were highly unsatisfactory. Subjects which had previously gone to sleep within a few minutes of their entrance into the animal chamber were observed, when in the hyperthyroid state, making repeated attempts to compose themselves, but always failing. The basal metabolism determinations were therefore made under light nembutal anesthesia (40 mgm./kgm., ip.). This treatment depressed metabolism below that during deep sleep, in varying degree on different occasions, yet it gave a better comparison between normal and hyperthyroid values and was adopted as standard procedure.

Production of polycythemia. The finding of Waltner (10) that both the erythrocyte count and the hemoglobin value of rats' blood could be increased by cobalt feeding has been abundantly confirmed. This phenomenon was utilized in the present study by adding to the drinking water that amount of 10 per cent CoCl_2 which was calculated to afford each animal a daily intake of about half a milligram, in terms of the cobalt ion.

Since Orten *et al.* (11) showed a toxic effect of cobalt, and that it could be overcome by simultaneous feeding of manganese, an equal volume of 10 per cent MnCl_2 was used to supplement the cobalt, also by addition to the drinking water.

Production of anemia. The following method was developed for removal of large quantities of blood from rats. Under moderate nembutal anesthesia (50 mgm./kgm., ip.), the animal's tail was maximally vascularized by immersion in water at about 50°C . for about a minute. A two-inch length was then severed from the tail and the bleeding stump submerged in 5 per cent sodium citrate solution, preferably warm, to prevent coagulation. Usually, blood continued to spurt into the citrate for 10 minutes or more, and as much as $6\frac{1}{2}$ cc. could be removed thus from a 250-gram rat. Since the species has been estimated as possessing some $4\frac{1}{2}$ cc./100 grams body weight, this amounted to well over a 50 per cent depletion of its total blood volume.

By using a 25 cc. graduate cylinder previously charged with citrate to the 15 cc. mark, it was possible to control the quantity of blood removed by observing the level in the graduate and, at the desired time, arresting the hemorrhage by chilling under the cold water faucet.

Determination of hemoglobin. The method of Evelyn (12) was followed, except that the hemoglobin was oxygenated by shaking with 0.1 per cent Na_2CO_3 , rather than dilute NH_4OH , for a more stable oxyhemoglobin suspension. An Evelyn photoelectric colorimeter was used to determine the density of the blood- Na_2CO_3 mixture and the hemoglobin content calculated from the photometer reading.

EXPERIMENTAL RESULTS. In all experiments not otherwise specified, each rat underwent repeated determinations of heart rate and a single measurement, under nembutal anesthesia, of basal metabolic rate for establishment of its "predictable" values. After these standards were clearly attained, the particular experimental procedure was pursued for a period of 12 to 16 days, during which the changes in heart rate were followed and the terminal metabolic rate measured. Finally, the animal was sacrificed for analysis of cardiac glycogen, and comparisons were made with the normal and with the results of other procedures.

The alterations of basal metabolism obtained were taken as indicating the metabolic stimulation of the heart, on the basis of the observation by Dock and Lewis (13) that the rise in oxygen consumption of the rat heart-lung preparation paralleled that of the whole animal under thyroid administration.

Production of experimental hyperthyroidism. A moderate degree of hyperthyroidism was induced by feeding 25 mgm./100 grams/day of Armour's desiccated thyroid, blended into a small amount of salad dressing or grated carrot. Most animals consumed the mixture readily, especially with the latter vehicle; those few which refused were given the medication by force until the hyperthyroid state set in, after which they were nearly always so hungry as to require no further urging.

This single dosage level of thyroid substance, taken in all instances from a common bottle, exerted a highly inconstant effect on heart rate, metabolism and cardiac glycogen. The heart rates in 25 rats were accelerated by 5 to 50 per cent (average, 26 per cent); basal metabolism was increased in the 12 rats studied under nembutal anesthesia by 23 to 72 per cent (average, 51 per cent); and the hearts were depleted of their glycogen contents to a similarly variable extent (fig. 2).

Statistical handling of experimental and control data together did not prove advisable; the mere fact that all hyperthyroid animals varied in the same direction from the controls made several relations appear more real than closer scrutiny permitted. The correlation coefficient of the percentage increase in heart rate with the percentage increase in metabolism, determined according to the method of Fisher (14), gave an r value of 0.7799, corresponding to a probability of well under one chance in one hundred of the relation being fortuitous. But similar analysis of the experimental values alone obtained an r of -0.4649 , obviously without meaning, especially in view of its negative sign (see fig. 2a).

Correspondingly, trend lines, computed by the method of least squares, were distorted by the inclusion of control values. In figure 2, therefore, two such trends were constructed for each graph, one including control and experimental data (broken line), the other depending on experimental figures only (solid line).

The nearness with which those two trends coincided was taken as indicative of the innate relation between the factors involved.

As illustrated in figure 2, there was no close relation between the stimulation of heart rate and that of metabolism in hyperthyroidism (graph *a*), nor between the percentage increase in metabolic rate and the decrease of heart glycogen (graph *b*). On the other hand, 25 rats, including the 12 used in relating glycogen

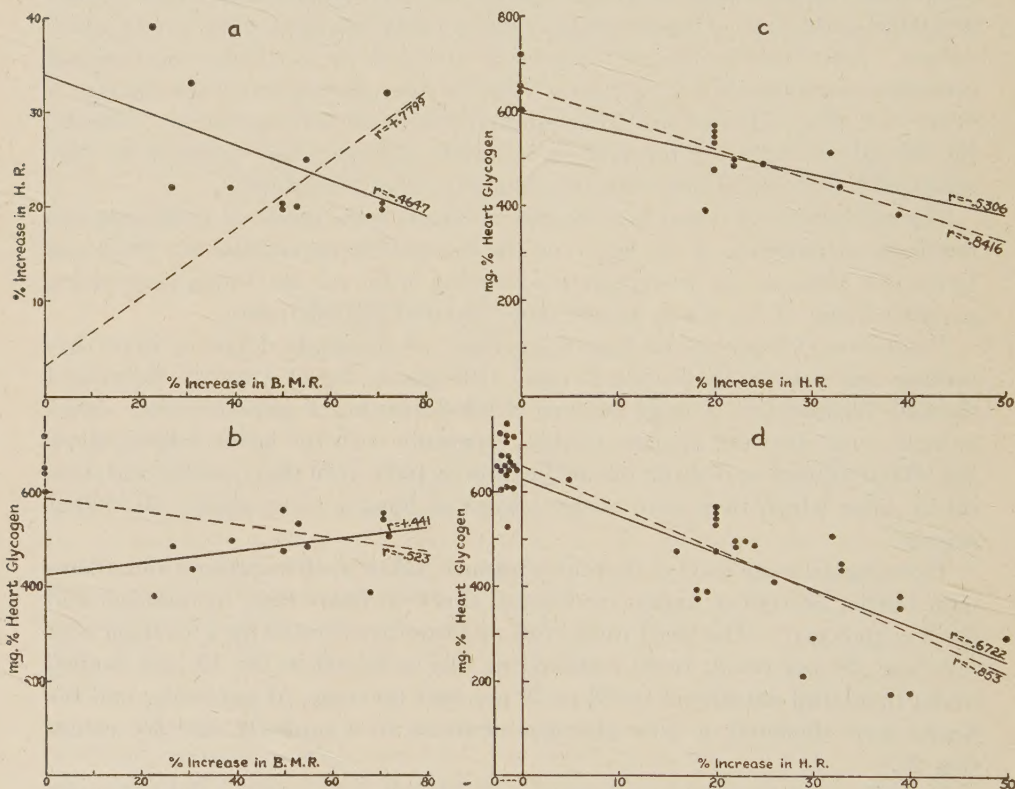


Fig. 2. Effect of hyperthyroidism. Solid trend lines are computed from experimental rats only; broken trend lines are from experimentals and controls, combined. Correlation coefficients based on experimentals only are placed at ends of solid trend lines; correlation coefficients derived from experimentals and controls are at ends of broken trend lines. *a*, relation of heart rate increase to metabolism increase; *b*, relation of heart glycogen to metabolism increase; *c*, relation of heart glycogen to heart rate increase (same individual rats as in *a* and *b*); *d*, relation of heart glycogen to heart rate increase (controls of entire series are placed at 0—0).

to metabolism, showed that the decrease in heart glycogen roughly but definitely paralleled the percentage increase in heart rate (graphs *c* and *d*).

Deceleration of hyperthyroid heart. Several attempts were made to depress the rate of the hyperthyroid heart to or toward normal. These included the intraperitoneal injection, twice each day, of as much as 6 cc. (1.2 mgm.) of lanatoside

C², 0.3 mgm./100 grams of thevetin³, 0.02 mgm./100 grams of carbaminoylcholine chloride, and 15 mgm./100 grams of quinidine sulphate. In no instance was the pulse retarded; in some individuals, an even greater tachycardia was produced.

None of the data assembled on these animals was incorporated into any graph, since, in the face of the atypical effect on heart rate, their actions on other phases of cardiac physiology could not be deduced from the literature. However, the glycogen values obtained were such that they could have been inserted without distortion into the graph relating glycogen decrease to the degree of tachycardia (fig. 2*d*). That is to say, administration to hyperthyroid rats of none of these agents had any significant effect on either the tachycardia or the depression of heart glycogen.

Cardiac acceleration by non-thyroid means. Chilling. Subjection of rats to increased thermoregulatory demands was accomplished by depilation with saturated BaS of the entire body below the head. Some of the rats were subsequently placed in an environmental temperature of about 20°C., some in the refrigerator at 2°C. (all these animals died), and some were kept at 27°C.

According to the findings of Hamilton, Dresbach and Hamilton (15) and of many other investigators, a depression of body temperature and of heart rate, along with an elevation of metabolism, were expected to occur on thus chilling the animals. Actually, probably because no restraint was exercised upon the rats during these relatively prolonged experiments, an increase in heart rate obtained in the five hyperthyroid, normal and thyroidectomized rats which survived the several maneuvers.

Plotting the tachycardia thus produced against the terminal heart glycogen values gave the curve reproduced in figure 3*a*, to all appearances identical with that derived in hyperthyroidism alone; the trend line from figure 2*d* has been inscribed on this and other graphs for comparison. That the glycogenolytic effect was not exercised through a stimulation of thyroid activity was shown by 1, its occurrence in the thyroidectomized animals, and 2, the evidence of thyroid histology in the chilled normal and thyroid-treated animals. Hematoxylin-eosin sections from the chilled normal rats showed predominantly a flat acinar epithelium and colloid-filled alveoli, with a small proportion of partially empty alveoli having low cuboidal lining cells. The thyroid gland of the thyroid-fed rat which had been chilled exhibited only colloid-swollen acini surrounded by flat epithelium. Apparently, there was no stimulation of thyroid activity by the moderate chilling produced. The rectal temperature was reduced by about 1.5°F.

The completeness of thyroidectomy was vouched for by a definite fall in both heart rate and metabolism and by an absence of macroscopically identifiable thyroid tissue at autopsy.

Atropine. Atropine sulphate was administered by intraperitoneal injection of 10 mgm./100 grams, twice daily. This procedure was found to maintain the heart rate at levels approximating those obtained in the thyroid feeding experi-

² Lanatoside C (Cedilanid) was supplied by courtesy of Sandoz Chemical Works, Inc., New York.

³ Thevetin was supplied by courtesy of Eli Lilly & Co., Indianapolis.

ments, though the pulse probably began to regress toward normal during the hour or two preceding the morning injection. Since the dosage required was quite close to lethal, several animals died before completion of the course of injections; seven survived to the end.

The relation between cardiac acceleration and glycogen, expressed by the usual graphic method in figure 3b, roughly followed the same course as in the hyperthyroidism experiments.

Caffeine. As a means of stimulating the myocardium directly, caffeine citrate was given intraperitoneally in twice daily amounts of 8 to 15 mgm./100 grams. The smaller dosage was effective during the first few days of treatment, but development of tolerance by the animals required that the quantity be increased progressively. Maintenance of any constant degree of tachycardia was relatively unsuccessful and fluctuations therefore unavoidable.

As indicated in figure 3c, the depression of cardiac glycogen in the five rats which completed the experiment was clearly less than that which might have been expected from the levels of tachycardia developed. That difference might have been due to a simultaneous improvement of blood supply to the myocardium, resulting from the actions of caffeine in increasing general blood pressure and dilating the coronary vessels.

Ephedrine. Attempting to test the suspected cause of caffeine's difference from the other cardio-accelerators used, ephedrine sulphate was administered to one litter, alone or after production of hyperthyroidism. The maximum dose tolerated, 6 mgm./100 grams, ip., twice a day, produced relatively a slight speeding of the heart's action, followed after about six hours in most animals by a fall to, or even below, the control pulse level.

Of the five surviving rats, three were hyperthyroid. Two of these animals showed a decrease in heart rate, after the early rise, to somewhat below their hyperthyroid level, and their heart glycogens were 330 and 335 mgm. per cent, probably most nearly related to the degree of tachycardia they would have had with thyroid alone. The pulse rate of the other hyperthyroid individual was depressed to its control level, namely, the level before thyroid treatment was instituted; that animal's glycogen value was 523 mgm. per cent. Of the two non-hyperthyroid rats, one received a slight tachycardia from the ephedrine injections, while the other rat's heart rate was unchanged or slightly reduced; their heart glycogen concentrations were 403 and 692 mgm. per cent, respectively.

The consequences of ephedrine administration, thus, proved variable on both heart rate and heart glycogen, and no conclusions could be drawn from that experiment. The literature, too, has shown such a diversity; both an increase and a decrease in heart rate and blood pressure have been reported for unanesthetized animals by Chen and Schmidt (16).

Alterations in oxygen supply to the heart. The results obtained with caffeine administration, along with the well-known effect of anoxia in depressing heart glycogen (3), pointed to the possibility that the loss of glycogen in hyperthyroidism might be due to a hypoxia, depending on a relative anemia of the excessively

active heart. A method of testing this hypothesis was offered by studies on polycythemia and anemia.

Anemia. Five rats were bled by the method described, and their cardiac glycogen and hemoglobin values were determined five days later. The relation of heart glycogen to hemoglobin (fig. 4a) showed a decline in glycogen beginning at a hemoglobin level of 11 or 12 grams per cent and falling off rapidly thereafter.

The normal hemoglobin value, determined in six untreated rats, was 16.5 grams per cent; range, 15.4 to 17.4 grams per cent.

Polycythemia. Of a total of 23 cobalt-treated animals, 16 were rendered hyperthyroid and, with their 7 polycythemic controls, studied in the usual manner. The relation between heart rate increase and glycogen content (fig. 4b) showed a possible trend toward conservation of cardiac glycogen, especially at the higher

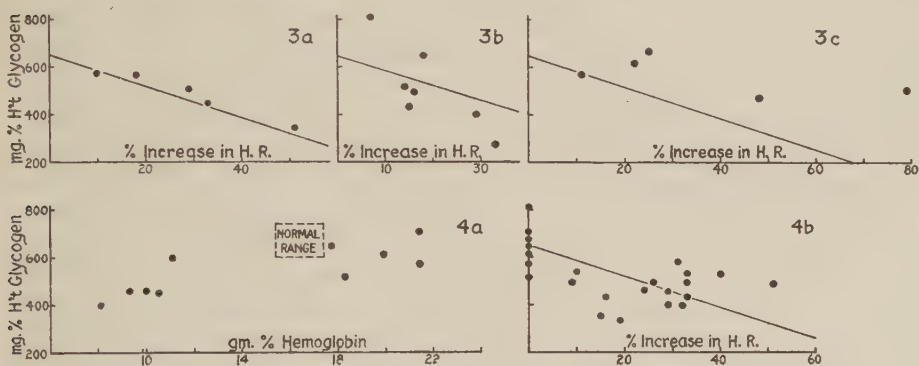


Fig. 3. Effect on heart glycogen of tachycardia produced by non-thyroid agents. Superimposed on each graph is the trend line obtained in figure 2d, relating heart glycogen to percentage increase in heart rate by thyroid treatment. a. Effect of chilling by depilation with barium sulphide. b. Effect of atropine. c. Effect of caffeine.

Fig. 4. Influence on heart glycogen of blood hemoglobin level. a. In non-hyperthyroid rats. Anemic rats at left, polycythemic at right, normal range as indicated. b. In polycythemic hyperthyroid rats. Polycythemic non-hyperthyroid controls are placed on ordinate guide line. Trend line from figure 2d is superimposed.

levels of tachycardia. The range of hemoglobin in 11 of these hyperthyroid polycythemic rats, the only ones on whom determinations were made, was 16.2 to 21.4 grams per cent.

The conclusiveness of this experiment is not overwhelming. Among those hyperthyroid polycythemic animals whose hemoglobin values were obtained, there was evinced no clear-cut protective trend; unfortunately, the four rats whose heart glycogens were highest above the expected were not among those whose hemoglobin levels could be determined. On the other hand, as indicated by the polycythemic control glycogens (fig. 4, a and b), the range of heart glycogen values in non-thyroid-treated animals was extended to limits of at least 811 and 516 mgm. per cent, implying a separate, variable influence of the cobalt treatment itself. Aside from this, there could be no assurance that the necessar-

ily increased viscosity of the polycythemic blood did not counterbalance any beneficial effect of the elevated oxygen carrying power.

Relative depletion of right and left ventricles. Another avenue of attack was offered by the fact that coronary blood flow to the musculature of the left ventricle is interfered with during systole. The deeper branches of these arteries have been demonstrated to be subjected to high external pressure by the contracting heart wall, thus impeding flow and relegating it mainly to the diastolic phase of the cardiac cycle. It has also been amply shown that an acceleration of the heart occurs primarily at the expense of the recovery period, so that any abbreviation in the cardiac cycle takes place as an encroachment on diastole, affecting systole but little. Hence, the proportion of the cycle during which there is free coronary flow to the subendocardial portion of the left ventricle must be less in a rapid than in a slow heart.

On these grounds, it seemed likely that, if the loss of heart glycogen during prolonged tachycardia is accountable to deficient blood flow—i.e., relative anoxia—to the heart musculature, the depletion would be correspondingly greater in the left ventricle. Comparative determinations were therefore attempted on the right and left ventricles, separately, in hyperthyroid rats. The glycogen analysis, however, proved far less reliable with the small amounts of tissue here involved, especially in the instance of the right ventricle, than those performed in the other experiments. The time elapsing between opening of the chest and immersion of the heart in boiling Ringer's solution became extremely important. Most consistent results, though still far from being completely satisfactory, were obtained when this "removal time" was held under two seconds and when the amount of left ventricle used for analysis was limited to less than twice the weight of the right ventricle. The values obtained on the 16 normal and 19 hyperthyroid hearts which accorded with these requirements (table 1) showed a generally higher glycogen concentration in the left ventricle in normal hearts and in the right ventricle in hyperthyroidism. The apparent loss of glycogen, upon moderate thyroid medication, by the right ventricle was 13 per cent, by the left ventricle 18 per cent, amounting to a 37 per cent greater loss by the left ventricle than by the right.

This evidence apparently favors the theory that the glycogen loss is attributable to a relative ischemia, exaggerated in the left ventricle by the dynamics of coronary circulation in that chamber. However, statistical analysis of these data showed that the significance of the difference between the means of control and experimental glycogens in the right ventricle had a t value of 3.04, of the left ventricle 5.95. The difference, though suggestive, is thus not highly significant.

DISCUSSION AND CONCLUSIONS. The observation that hyperthyroidism, chilling and atropinization all decrease cardiac glycogen, depending directly on the degree of tachycardia, seems to indicate that that effect is directly and causally associated with the increase in cardiac activity. The influence of cooling and of atropine are probably alike exerted predominantly or entirely through the nervous system, giving as nearly a pure acceleration as could be established in chronic animals, yet their effects on the glycogen level are as great as that of thyroid feeding.

These observations indicate that the glycogen depletion, at least, of the so-called "thyroid" heart is not due to any specific action peculiar to the thyroid hormone. Were it to be found possible to prevent or inhibit the tachycardia of hyperthyroidism, a more crucial test of the apparent relation to cardiac activity would be feasible.

The lessened glycogen depletion obtained with caffeine administration implies a major rôle on the part of some additional factor. That this factor may be one of blood supply to the heart wall is suggested by caffeine's action of increasing

TABLE 1
Comparative depletion of glycogen in right and left ventricles

CONTROL			HYPERTHYROID		
Right ventricle	Left ventricle	R.V. minus L.V.	Right ventricle	Left ventricle	R.V. minus L.V.
357	498	-141	466	441	+25
709	645	+64	580	521	+59
528	556	-28	425	480	-55
518	602	-84	426	424	+2(=)
579	567	+12	423	523	-100
638	650	-12	411	448	-37
640	669	-29	496	472	+24
436	473	-37	541	508	+33
537	535	+2(=)	482	465	+17
470	518	-48	451	568	-117
643	683	-40	519	442	+77
506	543	-37	602	528	+74
617	567	+50	478	443	+35
590	590	0(=)	496	424	+72
581	591	-10	465	439	+26
568	550	+18	557	511	+36
			459	456	+3(=)
			468	471	-3(=)
			435	450	-15
Av.: 558	577	-19	Av.: 483	474	+9

	R.V. > L.V.	R.V. < L.V.	R.V. = L.V.
Control.....	4	10	2
Hyperthyroid.....	12	5	3

cardiac circulation by raising the general blood pressure and dilating the coronary arterioles. Partial support for this explanation derives from the loss of heart glycogen brought about solely by severe anemia, and, contrariwise, by the tendency toward protection by polycythemia.

The theory may be advanced, quite tentatively, that a reduction of glycogen stores in the heart is consequent upon an ischemia to the myocardium. An increase in cardiac activity, beyond the limit of a parallel increase in blood supply under the particular conditions, must result in a relative anoxia. In the tachycardia of hyperthyroidism, some reflex and chemically stimulated adjustment of

circulation is doubtless made, but it is quite possible that the coronary dilatation which does occur is able to obtain insufficient additional blood flow in the face of more forceful demands by other hypermetabolizing tissues. The major driving force for coronary blood flow—aortic pressure—is not significantly augmented in hyperthyroidism.

This concept of an ischemic etiology of the glycogen depletion accords well with the ideas advanced by Goodpasture (1) and by Simonds and Brandes (17) in their attempts to explain the microscopic appearance of the "thyroid" heart. Similar cardiac histopathology to that claimed specific for hyperthyroidism was described by Thorel (18) for patients with coronary insufficiency and by Schmidt-mann (19) and Pfeleiderer (20) for sections prepared from the hearts of rats, guinea pigs and rabbits in which coronary disease had been experimentally produced. The fatty and hyaline degeneration, round cell infiltration and scar tissue formation, frequently perivascular, which have been described as characteristic of thyroid intoxication are precisely what one should expect from myocardial ischemia.

A definitive statement cannot, however, be made until actual studies of coronary arterio-venous oxygen differences have been carried out. Certainly the finding of Essex *et al.* (21) that the coronary flow may be increased in hyperthyroid dogs by a maximum of about 200 per cent does not support such a concept; it would strongly controvert this idea were that maximum maintained over a period of days or weeks.

The increased rate and work of the rapid heart are coupled with a necessarily abbreviated recovery time between beats, as well as a diminished period during which circulation is at its maximum, and these forces may well set into motion a vicious cycle. Whether they actually do so must be determined by direct experimental analyses, preferably simultaneously, of these several factors.

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SUMMARY

1. Methods were developed for accurate and consistent determination of heart rate, oxygen consumption and cardiac glycogen in the white rat. A technique was also devised for the removal of large amounts of blood from the rat's tail.

2. Standard "predictable" levels of heart rate and basal metabolic rate were established in 110 rats. After approximately two weeks' experimental manipulation, cardiac glycogen contents were determined and compared, in relation to the changes produced in heart rate and metabolic rate, with normal controls and with each other.

3. Moderate hyperthyroidism was found to deplete heart glycogen to an extent directly related to the increase in heart rate but not closely related to the metabolic stimulation.

4. Atropine and chilling (by depilation) decreased heart glycogen, in relation to the tachycardia produced, to a degree similar to that obtained in hyperthyroidism.

5. Caffeine caused a smaller loss of heart glycogen, with reference to the stimulation of heart rate, than would have been predicted from the effects of thyroid, atropine and chilling. This might have resulted from the increased blood pressure and the coronary dilatation characteristic of caffeine medication.

6. Primary anemia produced a decrease in heart glycogen roughly correlated with the decline in hemoglobin content of the blood. Cobalt polycythemia seemed, to a slight extent, to protect the hyperthyroid heart from loss of its glycogen stores.

7. The decrease in heart glycogen of 19 hyperthyroid rats was participated in by both the left and right ventricles. Loss by the left ventricle was greater by an average of 37 per cent, but the variability of results, due to technical difficulties, renders this difference not statistically as significant as might be desired.

It is concluded that the decrease in heart glycogen by hyperthyroidism is not due to any specific "toxic" influence of the thyroid hormone. The suggestion is tentatively offered that the glycogenolytic action is exerted through a relative ischemia caused directly and indirectly by the increased cardiac activity.

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